STUDIES ON THE BIOSYNTHESIS OF HOMARINE AND THE CATABOLISM OF PICOLINIC ACID IN *HOMARUS AMERICANUS* AND *BUSYCON FULGAR*

By

LOUISE NIYING CHU

Bachelor of Science
Taiwan Provincial Chung-Hsing
University
Taichung, Taiwan
1957

Master of Science
Texas Woman's University
Denton, Texas
1963

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1970

STUDIES ON THE BIOSYNTHESIS OF HOMARINE AND THE CATABOLISM OF PICOLINIC ACID IN HOMARUS AMERICANUS AND BUSYCON FULGAR

Thesis Approved:

Robert K. Gholson
Thesis Adviser

Patrick E. Geiss

D. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author wishes to acknowledge with her sincere appreciation to her major adviser, Dr. Robert K. Gholson, whose thoughtful guidance, advice, great patience and encouragement made this thesis possible. Special appreciation is extended to George V. Odell for his attention and advice.

Thanks are due to Dr. J. L. Chandler for his valuable suggestions and discussion during the last year of her research. Appreciation is also due to fellow member of the research group of Dr. R. K. Gholson for their help.

Sincere appreciation is extended to Dr. P. E. Guire for his help with Amino Acid Analysis.

Acknowledgment is given to Dr. G. R. Waller and Mr. Keith Kinneberg for the Mass Spectra Data.

The author is indebted to the Oklahoma State University Department of Biochemistry for providing research facilities and finanical assistance during this study.

Special gratitude is extended to her parents, Ying-Song and Han-Fong Chu, for their constant encourgement and endorsement during the years of graduate study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Since the discovery of homarine in the lobster in 1933 (1), it has been found that this compound occurs widely and in high concentrations in marine invertebrates. The concentration of this compound varies from species to species and also varies with the organ and season in the same species.

Homarine is a quaternary nitrogen base with interesting characteristics that suggested a possible role in nerve function. Furthermore, the absence of homarine in fresh water invertebrates suggested that this compound may be functioning as a cellular osmoregulator in marine organisms (2). However, these postulations were later disproved (3, 4). The unusual ease of decarboxylation of homarine compared to structurally similar compounds such as trigonelline and N-methylisonicotinic acid and the poorly developed circulatory system of invertebrates suggested that homarine could be involved in the excretion of carbon dioxide (5). The low concentration of this compound in the blood and urine indicated that it may be a metabolic end product (6). In spite of the considerable literature on homarine, no investigations of the biosynthesis

or metabolism of this compound have been reported and no substantive information is available to establish the physiological function of the compound.

Picolinic acid is considered as an end product of tryptophan metabolism in mammals. In the bacteria, however, picolinic acid is further metabolized to 6-hydroxy-picolinic acid (7). There are no reports at this time concerned with the picolinic acid metabolism in invertebrates.

This dissertation is concerned with studies on the biosynthetic pathway to homarine and possible metabolites of picolinic acid in lobsters and snails. L-aspartic acid-U-^{14}C, L-lysine-2-^{14}C, quinolinic acid-2, 3, 7, 8-^{14}C, D, L-tryptophan-benzene-U-^{14}C, acetate-2-^{14}C, glucose-U-^{14}C, methinonine-CH_3-^{14}C, Na-formate-^{14}C, picolinic acid-COOH-^{14}C, N-methylpyridine-CH_3-^{14}C, were injected into live lobster or snail muscle in separate experiments to determine whether or not these compounds were the precursors of homarine. The metabolic products were also determined following picolinic acid-COOH-^{14}C injection.

A column chromatographic method was developed to isolate the radioactive peaks of homarine of perchloric acid tissue extract and the peaks were determined by paper chromatography. The unknown metabolite which results from picolinic acid injection into lobster muscle was also isolated and characterized by column chromatography.

CHAPTER II

LITERATURE REVIEW

Discovery and Proof of Structure

Homarine was first isolated in 1924 from the urchin Arbacia lixula by Holtz, Kutscher, and Thielmann (8), but was considered to be trigonelline by these authors. The compound was also isolated from the jelly fish Velella spirans (9) and the lamellibranch Arca noa (10) but was still identified as trigonelline. In 1933, Hoppe-Seyler (1) isolated the same compound from the lobster Homarus vulgaris, and the sea urchin Arbacia pustulosa. From differences in the melting point, the pine-shaving reaction which distinguishes α from β and γ -pyridine derivatives, and the melting points of derivatives, he identified this compound as the methyl betaine of picolinic acid and named it "homarine".

Distribution of Homarine

Following the structure proof of homarine, this compound was detected in the tissues of many marine and brackish water invertebrates. Fresh water invertebrates and vertebrates, however, contained no detectable amounts

of this compound.

In 1942, Mii (11) found homarine in the lobster, Penaeus joyneri, in a quantity of 0.05 per cent of total lobster muscle.

Homarine was also isolated from other marine invertebrates, such as the coeleterates, condylactis, physalia, metridium, and plexaura (12); sea anemones, Calliactis parasitica, Actinia equina, Anemonia sulcata and Metridium senile (13); marine gastropods, Mures fulvescens, Urosalpinx cinereus, and Thais lapillus (14); also in some Australian marine crustacea, such as the Moreton Bay lobster Thenus orientalis, king prawn Penaeus plebejus, tiger prawn Penaeus esculentus and spiny crayfish Jasus verreauxi (15). The compound was also found to be present in abalone (16).

From 1949 to 1961, Ackermann and co-workers (17, 23, 24, 25, 26, 27) identified homarine, and often trigonelline, in the tissues of the sea anemone, Arca sulcata, the sea snail Patella sp., the marine snail, Arenicola marina, the crab Crangon vulgaris, the king crab, Limmuls polyphemus, the leather coral, Alcyonium digitatum, the sponge, Calyx nicacensis, and the fungus, Polyporus sulphureus.

Kalckar, Strominger and Gevirtz (26) isolated homarine from the foot muscle of Busycon canaliculatum. They reported that homarine has a maximum absorbance at 272 mu which did not change between pH 0 to pH 14. The

compound was absorbed strongly on Dowex-50-H^+ and could be eluted with 2 N hydrochloric acid. It was not absorbed on Dowex-2-acetate.

Using ultraviolet absorption, Hultin et al. (27) could determine the homarine content quantitatively in the sea urchin eggs and embryos of four species, Arbacia lixula, Sphaerechinus granularis, Psammechinus microtuberculatus, and Echinis esculentus. They found that the homarine content in unfertilized eggs varied among the species, but that the concentration did not change significantely during the course of normal development. Deffner and Hafter (28) also found slight differences in the content of this compound in the muscles of various marine invertebrates. They found that Loligo pealii and Dosidicus gigas contained 2.92 and 3.14 per cent homarine respectively by weight. The differences in homarine content between Thais and Urosalpinx were more obvious in the report of Keyl et al. (14). In their experiments, they found Thais lapillus contained 2.6 milligrams of homarine per gram of body weight, whereas Urosalpinx cinereus only contained 1.7 milligrams.

Koechlin (29) reported that the concentration of homarine in squid varies considerably with the season. The absorption at 272 mu of undiluted axoplasm was higher in May and June, whereas the concentration of homarine dropped to one half of this value in August and September.

In 1955, Gasteiger, Hergen and Haake (2) carried out a thorough study on homarine distribution in the animal kingdom. They concluded that in the phyla Porifera, homarine content was less than 0.2 milligrmas per gram of tissue, (wet weight) whereas the Mollusca contained 7.7 milligrams and Coelenterata 0.6, Ctenophora 0.2, Annelida 1.1, Echinodermata 0.3, and Arthropoda 8.7 milligrams per gram of tissue (wet weight), respectively. Within the phylum Mollusca, Venus and Pecteu tissue content was considerably below than that of Loligo, Busycon and Nossa. Gasteiger and his co-workers (3) made a further investigation of Loligo, Homarus and Limulus in 1960 and they observed that homarine was widely distributed within the different tissues such muscle, nerve, and gill. In general, nerve and muscle showed very high concentrations, while the skin, mesentery, and stomach contained less. The lowest concentrations appeared in urine and blood. It was discovered (30) that in the giant axon of squid, the compound was unequally distributed along its length. Fukushima (31) found with a polarographic technique that homarine was more concentrated in the liver than in the arms and mantle in Ommatostrephes sloani and Octopus ochellatus.

Proposed Functions of Homarine

Since homarine was found high concentrations in nerve tissue, and had characteristics that suggest several possible roles in nerve function or muscular contraction,

Gasteiger et al. (3) performed perfusion studies with homarine using the lobster heart to test the possibility of these functions. However, they found no evidence that homarine functions in nerve conduction or muscle contraction. The absence of homarine in fresh-water organism suggested that homarine might serve as an osmoregulator. If this was true, the quantity of homarine should vary directly with the environmental salinity. This postulation, however, is not supported since there is a lack of correlation between the quantity of homarine in the nerve cord and the osmotic concentration of the environment (4). Therefore homarine does not appear to function to maintain the hyperosmoticity.

Riegel and his co-workers (32) observed that homarine which was isolated from the mussel shellfish, Mytilus californianus, was closely related to the mussel poison, but Schantz (33) later reported that the poison is saxitoxin which is not related to homarine.

Kravitz et al. (34) suspected that homarine might be acting by blocking nerve impulse transmission in the lobster and crab neuromuscular junction. However, this function seems unlikely since their results showed that only 0.14 per cent of this activity was contributed by homarine.

Tsukamoto and Komori (35) found that when toads were placed into a light-adaptation apparatus and the test compounds were injected into the lymph sac, homarine

could accelerate the visual function after 30 and 60 minutes for dark adaptation.

Fukushima (31) suggested a possible role of homarine in a biological oxidation-reduction system. Haake and Mantecon (5) stated, on the other hand, that the unusually large quantities of this compound in invertebrates seemed to be far more than would be required for a coenzyme of the NAD type to which there is some structural analogy.

Homarine was found to decarboxylate about 10^3 times faster than the other position isomers, trigonelline and N-methylisonicotinic acid. This phenomenum lead Haak and Mantecon (5) to hypothesize that the developed circulatory systems of invertebrates required a carbon dioxide sink to store their metabolic product during the period of activity and that homarine might result from carboxylation of the N-methylpyridinium ion, providing such a carbon dioxide storage.

Possible Biosynthetic Pathways to Homarine

Although the function of homarine in marine organisms still remains obscure, it is probably an important compound in these animals since it is present in such large quantity in the muscle and in various organs. There has been little work done so far concerning the possible metabolic pathways of homarine in marine invertebrates. Hoppe-Seyler (1) proposed lysine, arginine, citrulline,

ornithine, proline and glutamic acid, as possible precursors of homarine at the time he identified this compound.

Ciusa and Barbiroli (36) devised an *in vitro* method for determination of labile methyl groups based on irreversible transmethylation of nicotinamide to trigonellinamide with bovine blood. Their results showed that trigonelline was a methyl donor and some other compound was a methyl acceptor. They also found that a series of compounds such as choline formate, betaine and methionine were methyl donors and nicotine, pyridine, picolinic acid, and quinolinic acid were methyl acceptors.

In contrast to the results of Ciusa and Barbiroli, Mayer and Vigneaus (37) reported that trigonelline did not transfer its methyl group in the rat. Joshi and Handler (38) also had found that the methyl group of trigonelline underwent oxidative demethylation. The nicotinic acid so formed was used for NAD synthesis in yeast.

McKennis *et al.* (39) found, that essentially all the radioactive trigonelline was excreted in the urine and only 0.1-0.9 per cent of the radioactivity appeared in carbon dioxide after N-methyl-^{14}C trigonelline was injected into the rat.

From the above literature survey, if homarine were formed as the result of transmethylation, then, the possible methyl donors or the methyl acceptor compounds such as picolinic acid, formic acid, and methionine should be precursors of homarine.

Since the pyridine ring is the main structure of homarine, any compound which would lead to form the pyridine ring also could be a precusor of homarine. Griffith and Byerrum (40) found that in tobacco plants fed acetate -2-^{14}C or succinate-2, 3,-^{14}C, 72 per cent of the ^{14}C was associated with carbons 2 and 3 in the pyridine ring. Friedman and Leete's results (41), however, showed that only 37 per cent of the radioactivity appeared in carbons 2 and 3 after sodium acetate-2-^{14}C was injected into Nicotiana glauce.

Griffith et al. (42) fed glycerol-2-^{14}C and aspartic acid-3-^{14}C to Nicotiana rustica plants. They found that both of these radioactive compounds contributed relatively large quantities of radioactive carbon to the pyridine ring. Jackanicz and Byerrum (43) found that aspartate -3-^{14}C labeled carbon 3 of the pyridine with 57 per cent of the total radioactivity. Yang, Gholson and Waller (44) showed that glycerol was incorporated without randomization to carbons 4, 5 and 6 of the pyridine ring of nicotine in tobacco plants. They theorized that the incorporation of glycerol-^{14}C into carbons 2 and 3 of the pyridine ring probably resulted from its conversion to a 2-carbon unit which is then incorporated into a dicarboxylic acid which is the precursor of carbons 2 and 3 of the pyridine ring. They also found that almost all the radioactivity was located in the pyridine ring after quinolinic acid 2, 3, 7, 8-^{14}C was administered.

Ahmad and Moat (45) showed that yeast could utilize tryptophan-^{14}C uniformly labeled in the benzene ring under aerobic conditions to form nicotinic acid.

Possible Metabolic Pathways of Picolinic Acid

It is known that picolinic acid is formed by the action of liver enzymes on 3-hydroxyanthranilic acid. 3-Hydroxyanthranilic acid is an important intermediate in tryptophan metabolism and is known to be a precursor of nicotinic acid (47). In Mehler's work (6), however, it was found that most of the isotope of a small dose of carboxyl-labeled 3-hydroxyanthranilic acid given to rates appears as CO_2, whereas carboxy-labeled nicotinic acid does not yield labeled CO_2. This was interpreted as evidence that the decarboxylation to form picolinic acid is a major step in the metabolism of 3-hydroxyanthranilic acid *in vivo*. Picolinic acid thus formed was found to be essentially quantitatively as its glycine conjugate in the urine (6).

Dagley and Johnson (7) found that picolinic acid could be further oxidized to 6-hydroxypicolinic acid by *Aerococus* and *Rhodotorula*.

Dipicolinic acid was discovered to be a major constituent of aerobic spore forming bacteria by Powell (48). It was concluded that the biogenesis of this compound involves the condensation of pyruvate and aspartate to yield an unsaturated derivation of α-keto-ϵ-aminopimelic which then undergoes cyclization and oxidation (49,

50). In addition, N-succinyl- α amino- ε -ketopimelic acid had been established as an intermediate in the biosynthesis (51, 52). It has been shown that in Escherichia coli, pyruvate and aspartic semialdehyde condensed to form dihydropicolinic acid, which serves as an intermediate (the branch point) in the biosynthesis of lysine and diaminopimelate (53).

By using radioactive substrates, Tanenbaum and Kaneko (54) found that 1, 7-^{14}C diaminopimelic acid was transformed by P. citreo-viride into dipicolinic acid labeled essentially in its carboxyl groups, whereas aspartic acid -4-^{14}C appeared to be distributed in the ring and carboxyl groups of dipicolinic acid. Their results suggested that tricarboxylic acid cycle components are intimately related to dipicolinic acid biosynthesis. Extracts prepared from sporulating cultures of B. megaterium could carry out the net synthesis of dipicolinic acid when supplemented with pyruvate and aspartic semialdehyde (55).

There have been no reports concerning the further metabolism of dipicolinic acid or the possibilities of further degradation of picolinic acid.

CHAPTER III

EXPERIMENTAL PROCEDURE

Materials

Live lobsters, Homarus americanus were obtained from a local resturant in Oklahoma City, Oklahoma. Sea snails, the conch, Busycon fulgar, were obtained from New England Biological Associates Inc., Narragansett, Rhode Island.

The reference homarine was purchased from K & K Laboratories, Plainview, New York.

The D, L-tryptophan-benzene-U-^{14}C, glucose-2-^{14}C, lysine-2-^{14}C, formate-^{14}C, and barium carbonate-^{14}C were obtained from Nuclear Chicago. Glucose-U-^{14}C, Methionine-methyl-^{14}C, and sodium acetate-2-^{14}C were purchased from Amersham-Searle Corporation.

Quinolinic acid- 2, 3, 7, 8-^{14}C was a gift from Dr. G. Waller of Oklahoma State University.

Homarine-CH_3-^{14}C was synthesized by Bih-Jeng (56) for this study in this laboratory. The synthesis of homarine-CH_3-^{14}C .HCl was completed by heating alpha picolinic acid with CH_3I-^{14}C at 70-80°C for 18 hours. The product was crystalized from ethanol.

N-methylpyridine-CH_3-^{14}C was synthesized by the author in this laboratory using the methods for homarine synthesis.

Dowex-50 X 8, 200-400 mesh, cation exchange resin in the H^+ form was purchased from Sigma Chemical Company, St. Louis, Missouri.

Anion exchange resin, Dowex-1 X 8, 200-400 mesh, in the Cl^- form was purchased from Bio-Rad Laboratories, Richmond, California.

Silica gel thin layer plates were purchased from Quantum Industries in New Jersy.

Sephadex G-50 was purchased from Pharmacia Fine Chemicals Inc., Piscatway, N. J.

All other chemicals were of reagent grade and were obtained from local supply houses.

Picolinic Acid Synthesis

Picolinic acid-COOH-^{14}C was synthesized essentially as described by Schenk (57). To a solution of 12.56 millimoles of 2-bromopyridine in 10 ml of absolute ether was added drop by drop 14.17 millimole of butyl-lithium in 10 ml of ether with strong stirring and constant passage of dry nitrogen gas at -42°C. Then the reaction mixture was stirred for 5 minutes at -40°C. In a period of 2 minutes, it was cooled to -70°C and the system evacuated. Then the connection to the vacuum was closed and about 15 ml of concentrated H_2SO_4 were added to 4.35 millimole of $BaCO_3$-^{14}C. After 5 minutes, 4.0 ml of 3.5 N HCl was added to the solution. The reaction mixture was evaporated to dryness in the vacuum evaporator and dissolved in

5 ml of water and the picolinic acid preceipited with 1.5 gm of cupric acetate. For complete crystallization, the mixture should stand at 5°C for 20 hours. Free picolinic acid was regenerated by passing hydrogen sulfide gas into the copper complex which had been washed with acetone and dissolved in 0.5 N HCL. This partially purified compound was further purified on a 50 X 2.6 cm Dowex 50-H^+ column which was eluted stepwise with 300 ml of 1 N HCl, 300 ml of 2N HCl, and 750 ml of 3.5 N HCl. Fractions of 10 ml were collected and the absorbance of each fraction was determined at 268 mu in a Beckman DU Spectrophotometer. The tubes which contained picolinic acid which was eleuted with 3.5 N HCl were pooled and lyophilized. The picolinic acid was identified by the Fe $(NH_4)_2$ $(SO_4)_2$ color test described by Pollini (58).

For final purification, the compound was dissolved in water and passed over a 2 X 40 cm Dowex-1-formate column. The column was washed with 300 ml of H_2O and picolinic acid was eluted in 0.1 N formic acid. Fractions of 10 ml were collected and each fraction was determined at 268 mu as before.

The solution containing picolinic acid was lyophilized and the purity of the compound checked by paper chromatography. The radioactivity was determined with a Nuclear Chicago 4 Π strip counter. Mass spectra of the compound (Figure 1) were measured using a prototype (59) of the LKB 9000 combination gas chromatography-mass spectrometer instrument (Karolinska institute, Stockholm, Sweden).

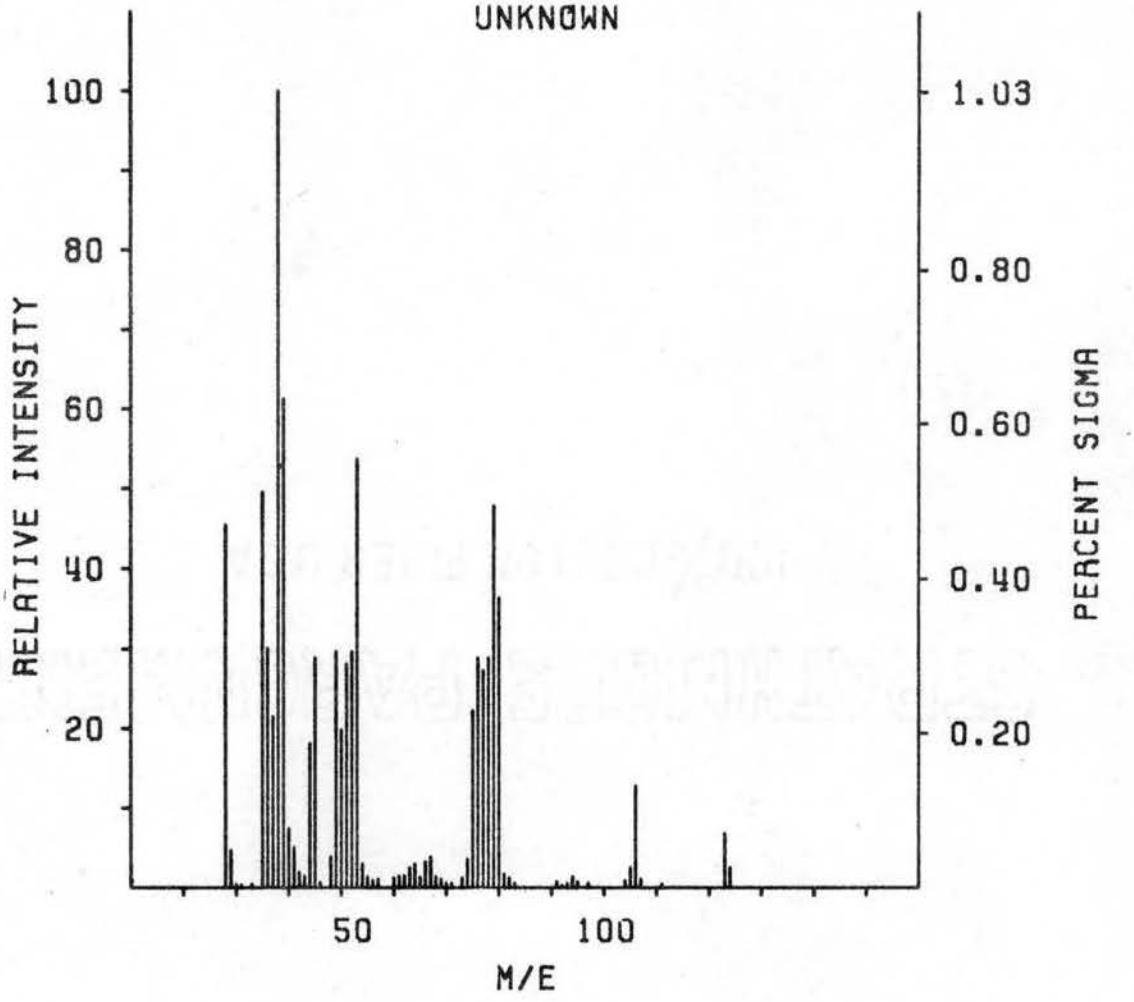

Figure 1. The Mass Spectrum of Synthesized Picolinic Acid

The sample in its solid state was introduced directly into the ion source through a direct inlet probe. The ion source temperature was 310°C, ionization current was 65 milliamps, multiplier voltage 2.1 kv, and acceleration voltage 3.5 kv. The mass spectra were computer plotted from tabular intensity data with a Cal Comp using a Fortran 11-D program. The mass spectra are reported in terms of relative intensity, the most abundant ion being taken as 100%, and in terms of sigma value. The spectra were computer plotted from tabular intensity data (60). For comparism of the purity of the synthesized compound, the mass spectrum of the recrystalized authentic picolinic acid from water was also measured as shown in Figure 2.

Standard Homarine Detection

The detection of standard homarine was carried out by the procedure of Badiei (61) using column chromatography. Dowex-50-H^+ resin was recycled with 1 N NaOH four to five times to the H^+ form. The washed resin was packed into a 50 X 2.6 cm column in 0.2 N HCl. Twenty mg. of standard homarine dissolved in 10 ml of 0.2 N HCl was applied to the column. The column was eluted with 300 ml of 0.2 N HCl, 300 ml of 1 N HCl, 450 ml of 2 N HCl and 750 ml of 3.5 N HCl. Fractions of 10 ml were collected and the ultraviolet absorbance at 268 mu of each fraction was determined with a Beckman DU Spectrophotometer. The chromatogram is shown in Figure 3. The fractions of the

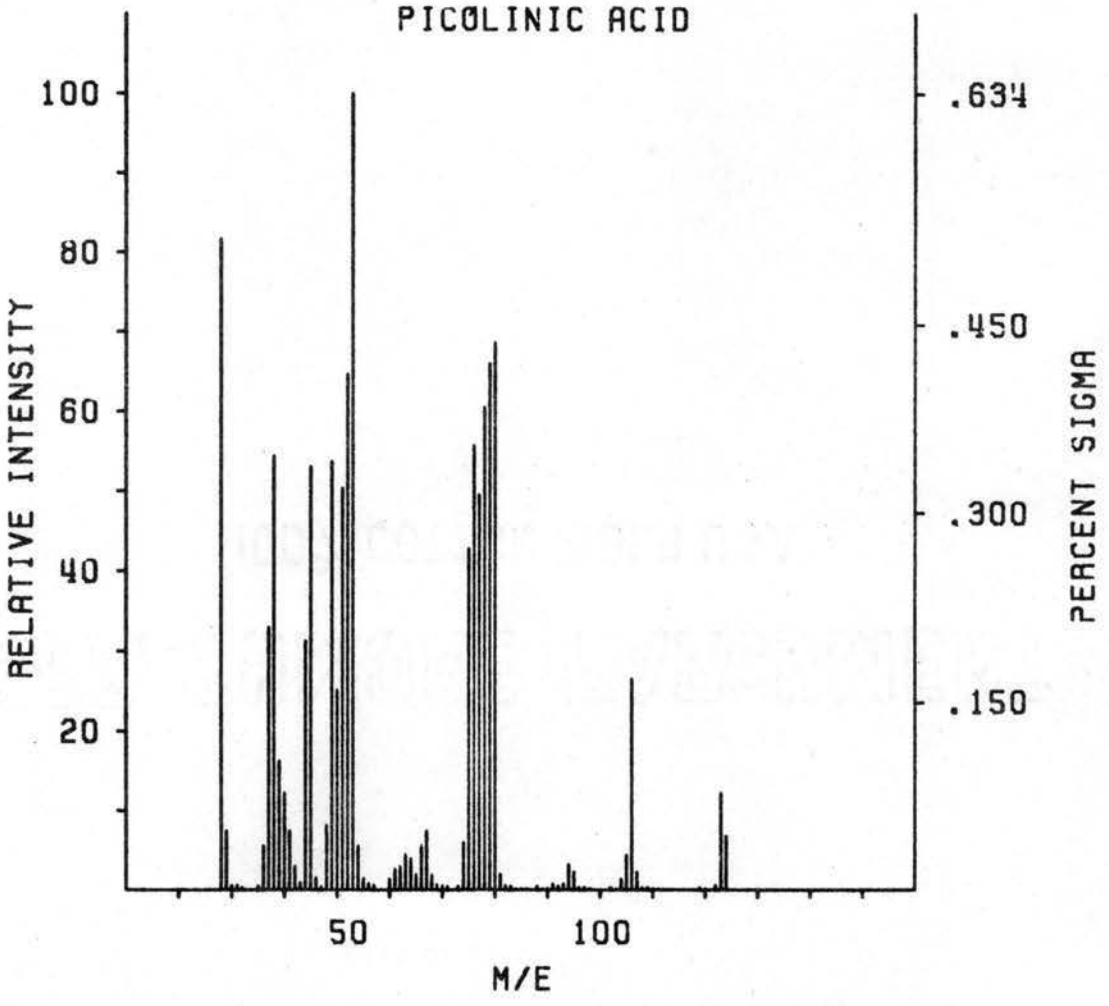

Figure 2. The Mass Spectrum of Authentic Picolinic Acid

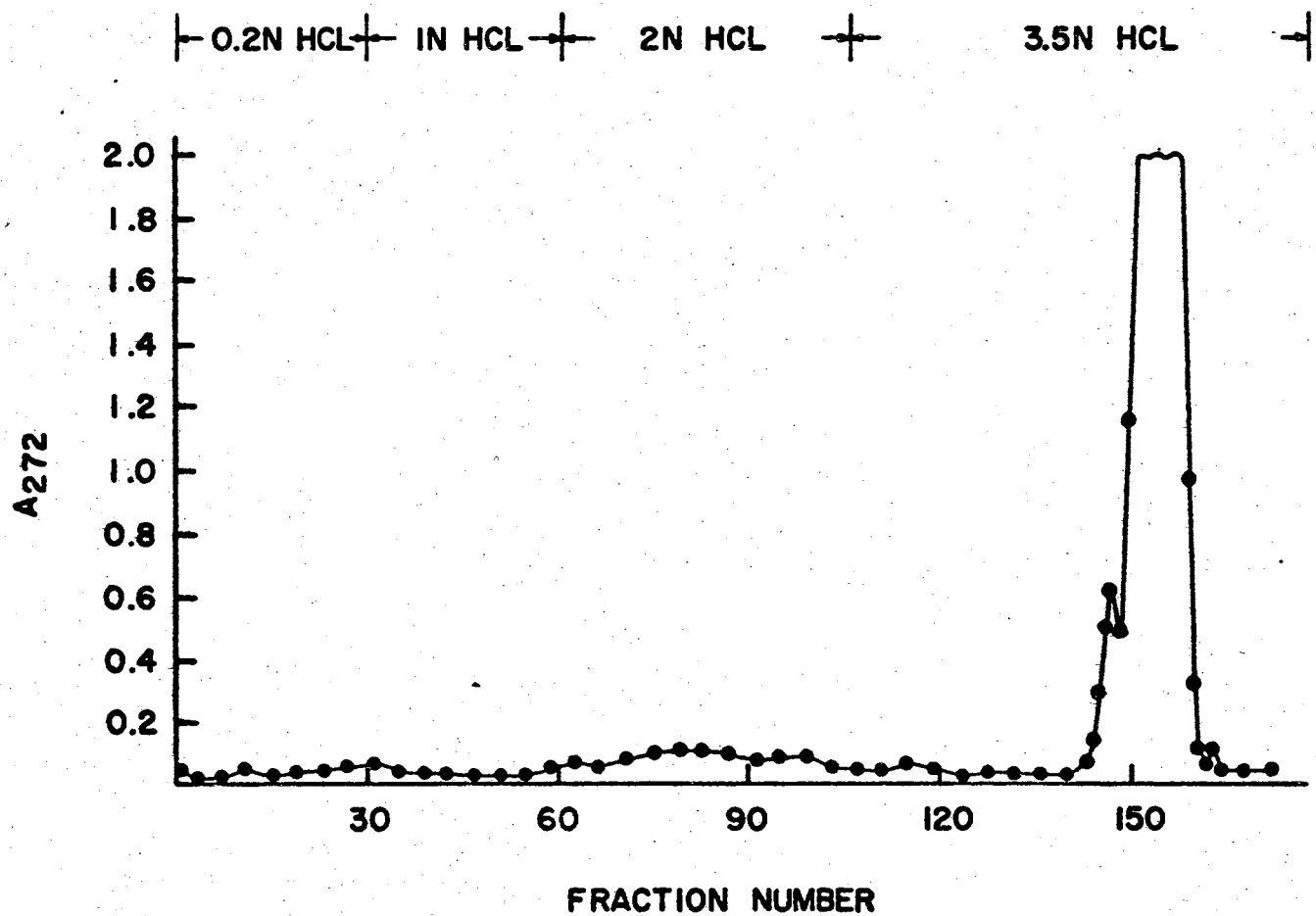

Figure 3. Chromatography of Standard Homarine on a Dowex-50-H^+ Column (50x 2.6 cm)

peak having maximum absorbance were pooled and evaporated to dryness in vacuo. The dried material was redissolved in 0.2 N HCl and spotted on Whatman No. 1 chromatography paper beside standard homarine, using 95 % ethanol: NH_4OH (95 : 5, v/v) as the developing solvent.

Animal Experiments

The lobsters and snails were kept alive in the cold room at about 4°C in artificial sea water which was prepared as reported by Prudden (62). It consisted of the following: 357 gm $NaCl.7H_2O$, 86.4 gm $MgSO_4.6H_2O$, 70.4 gm $MgCl_2.6H_2O$, 17.2 gm $CaCl_2.2H_2O$, and 9.1 gm KCl. The specified amounts were dissolved in 19.4 liters of tap water and compressed air was bubbled through the solution for few hours to remove chlorine. The bubbling was continued while the animals were kept in the water.

Isolation of Homarine from Marine Invertebrates

All lobster meat including chela and tail muscle was homogenized in two volumes of distilled water at top speed in a Waring Blender for 10 minutes and the mixture was made to 5 % perchloric acid. The mixture was centrifuged at 8,000 rpm. The residue was washed once with 300 ml of 5 % perchloric acid. The supernatants were combined and neutralized with 20 % KOH. The precipitate of $KClO_4$ was removed by centrifugation. The deproteinized muscle

extract was dried with a rotary evaporator. The dried extract was dissolved in 15 ml of 0.2 N HCl, the insoluble portion was removed by centrifugation and the supernatant solution was applied to a Dowex-50-H^+ column (50 X 2.8) and eluted stepwise with 0.2 N, 1 N, 2 N, and 3.5 N HCl. Fractions of 10 ml each were collected. The fractions were analyzed for 272 mu-absorbing material in a Beckman DU Spectrophometer. The elution-patterns are shown in Figure 4. The fractions corresponding to the homarine standard peak, (tube number 140-160) were pooled and evaporated to dryness on a rotarory evaporator. The dried material was redissolved in water and chromatographed beside standard homarine on Whatman No. 1 chromatography paper. The solvent systems used in the identification of the compound were as following:

t-Butanol : NH_4OH : H_2O (60 : 30 : 10, v/v/v)

n-Butanol : Acetic Acid : H_2O (73 : 10 : 17, v/v/v)

95 % Ethanol : NH_4OH (95 : 5, v/v)

Methods for Study of Homarine Synthesis in Lobster and Snail

The tail muscle of the lobster or the foot muscle of the snail was injected with the radioactive compound under investigation dissolved in the specified amount of water. Care was taken not to touch the nerve in the back of the lobster during injection.

The following radioactive compounds were injected into the invertebrates:

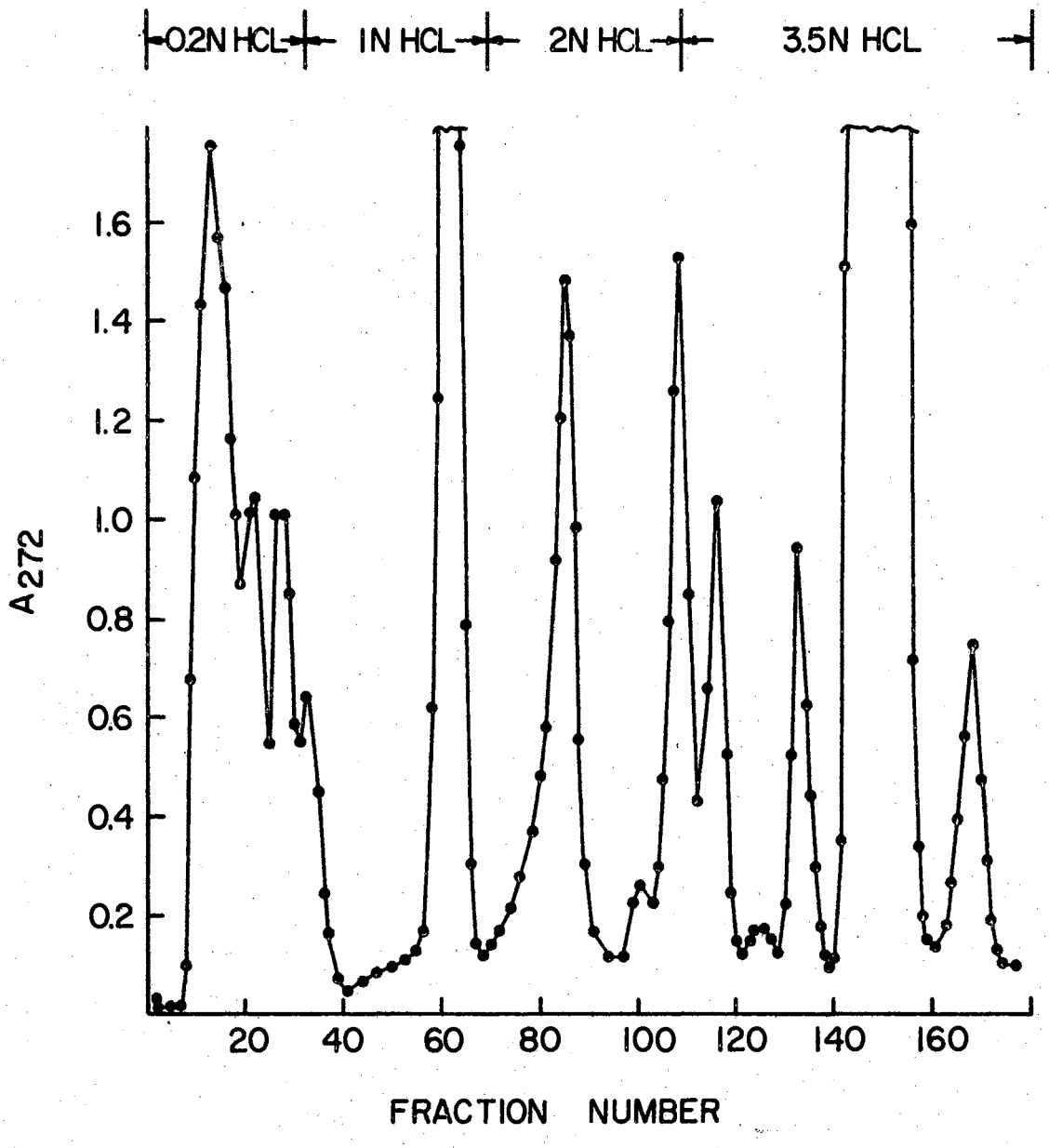

Figure 4. Chromatography of Perchloric Acid Extract of Lobster Muscle on a Dowex-50-H^+ Column (50 X2.6 cm)

Animal Radioactive Compound	Dose, Inj. (uc)	vol. (ml)
Lobster L-Aspartic Acid-U-^{14}C	24	0.48
Lobster L-Lysine-2-^{14}C	26	0.2
Lobster Acetate-2-^{14}C	11	0.2
Lobster D,L-Tryptophan-benzene-^{14}C	35	0.7
Lobster Quinolinic Acid-2,3,7,8-^{14}C	3.6	0.64
Lobster Glucose-U-^{14}C	50	0.75
Lobster Methionine-methyl-^{14}C	34	0.2
Lobster Sodium Formate-^{14}C	20	0.4
Lobster Picolinic Acid-carboxyl-^{14}C	2.56	0.8
Snail N-methylpyridine-methyl-^{14}C	2.5	0.2
Snail Homarine-methyl-^{14}C	2.5	0.5

After the injection was completed, the animal was kept in a dissicator containing 3 liters of artificial sea water. The dissicator was kept in a water bath to maintain the temperature between 15 and 18°C. Air was introduced through a sparger into the sea water in the dessicator and the respiratory CO_2 was collected by connecting the outlet of the dessicator to CO_2 absorption tubes. The 3 tubes contained 30 ml of ethanolamine in methycellosolve (1:2, v/v) (63). The final tube contained 300 ml of 2 N NaOH. The outlet of the final absorption trap was connected a water aspirator. To measure the radioactivity of the CO_2, 1.5 ml of the CO_2 trapping liquid was added to 7.5 ml

of methycellosolve-toluene (1:2, which contained 5.5 grams of PPO per liter and the radioactivity was determined in a liquid scintillation counter.

At the end of the experiment the animal was sacrified by putting it in a plastic bag into a -40°C deep freezer. The extraction and separation of the homarine peak was carried out as described above.

Each fraction eluted from the Dowex-50-H^+ column was monitored for radioactivity with a Packard Tri-Carb Liquid Scintillation Spectrometer, model 3950, by pipetting 0.1 ml aliquot into 10 ml of scintillation fluid. The scintillation fluid was composed of 600 ml of toluene, 400 ml ethanol, 4.0 gm of 2, 5-diphenyloxazole and 0.2 gm of 1, 4-bis (2-(5-phenyloxazolyl) -benzene (64). The efficiency of counting was periodially checked with a toluene-^{14}C standard supplied by the Packard Company and ranged from 50 to 54 %.

Detection and Purification of an Unknown Compound formed from Methionine in the Lobster

Anion Exchange Chromatography

The major radiocative peak obtained on Dowex-50-H^+ column chromatography of tissue extracts following methionine injection into the lobster was not free methionine. This compound was further purified by an anion exchange Dowex-1-formate column (1 X 29 cm) The resin was recycled 2 times with 4 column volumes of 2 N NaOH and 5

column volumes of HCl, followed by 10 column volumes of 6 N formic acid and finally with 2 N sodium formate to convert the Cl^- form to the formate form. The Dowex-1-formate column was thoroughly flushed with deionized water before the sample was applied.

The column was eluted with 300 ml of water, and 300 ml of 0.2 N formic acid. Fractions of 10 ml were collected. The absorbancy and the radioactivity of every fraction was measured.

Amino Acid Analysis

The radioactive compound eluted from the Dowex-1-formate column was lyophilyzed to dryness and analyzed for amino acid composition. The unknown compound was dissolved in 5 ml of 0.2 M citrate buffer and applied to the physiological column of a Beckman-Spinco Amino Acid Analyzer, model 120. The column was 0.9 X 56 cm and the compound was eluted by 0.2 M Na-citrate buffer of pH 3.25. The flow rate was 1 ml per minute. After 100 minutes, the Na-citrate buffer was changed to pH 4.26 with Na-citrate concentration of 0.38 M at 56°C.

For checking the possible amino acid composition of the unknown compound, an analysis of a standard known amino acid mixture was conducted under the same conditions as described above.

Since the amino acid analyzer could not detect radioactivity, another experiment under exactely the same conditions with the amino acid analyzer was conducted by using a small fraction collector attached to the physiological column and the solution eluted from the column was collected. Fractions of 2 ml were collected. This volume was equivelent to 2 minutes per fraction on the amino acid chromatogram. Radioactivity was determined in each fraction to distinguish which peak was metabolite derived from methionine-^{14}C.

Paper Electrophoresis

The sample was placed on a 40 X 3.7 cm Whatman No. 1 paper strip, 10 cm from one end. The paper strip was placed on an electrophoresis rack and spread with a buffer consisting of pyridine: acetic acid : H_2O (5.0 : 3.4 : 90, v/v/v), pH 5.0. The electrophoresis rack was placed in the electrophoresis tank, Savant, Model Ec-123 in the proper position and 2000 volts was applied for 40 minutes. 2-Propanol was used as the coolant. The initial temperature of the tank was 9°C and the final temperature was raised to 10°C. At the end of the experiment, the paper was monitored by paper strip counting.

Detection and Purification of an Unknown Compound Formed from Picolinic Acid in the Lobster

A total amount of 232.7 micromoles of radioactive picolinic acid which contained 2.56 microcurie was injected into the lobster as a possible precursor of homarine. The picolinic acid was dissolved in water and was injected into lobster muscle by the following technique: The first two injections of picolinic acid were made every twenty four hours with 0.48 uC/injection. The third injection of 1.6 uC was made twenty four hours after the second injection. The lobster was sacrificed forty eight hours after the third injection, so the experiment was ninty six hours in length.

The deproteinized lobster muscle extract was placed on the Dowex-50-H^+ column to determine whether or not picolinic acid is a precursor of homarine. Several radioactive peaks appeared upon Dowex-50-H^+ column chromatography. Fractions 151-158 were pooled and disignated as peak 2. Fractions 160-172 were pooled and designated as peak 3. Peak 2 and peak 3 were lyophilized individually to dryness and examined for the presence of homarine by paper chromatography. Three solvent systems were used for this purpose, sec-butanol : HCOOH : H_2O (5 :3 : 2, v/v/v), n- butanol : acetic acid : H_2O (73 : 10 :17, v/v/v) and t-butanol : NH_4OH : H_2O (60 : 30 : 10, v/v/v).

Fractions 20-27 were disignated as peak 1 and were pooled and lyophilized to dryness for further purification

and characterization. The above solvent systems also were used for paper chromatography of peak 1.

Anion Exchange Column Purification

The dried peak 1 from the Dowex-50-H^+ column was dissolved in 5 ml of H_2O and applied to a 2 X 38 cm anion exchange Dowex-1-formate column which was eluted stepwise with 200 ml of H_2O, 100 ml of 0.2 N formic acid, 150 ml of 1 N formic acid, 80 ml of 2 N formic acid, and 460 ml of 6 N formic acid. Fractions of 10 ml each for H_2O and of 5 ml each for formic acid were collected. The absorbance at 268 mu and the radioactive peak was pooled and lyophilized for further purification.

Thin Layer Chromatography

The compound obtained from Dowex-1-formate purification was dissolved in a small amount of deionized water and applied to a 15 X 4.8 cm silica gel thin layer plate. The plate was developed in n-butanol : acetic acid : H_2O (73 : 10 : 17, v/v/v). After developing and drying, the location of metabolites was determined with a paper strip counter and by ultraviolet light.

Chemical Analysis

The purified compound was tested with the $Fe(NH_4)_2(SO_4)_2$ reagent which is a specific test for 2-carboxy pyridine compounds (58).

The test for ninhydrin positive material was conducted by spraying a dried unknow spot with 0.2 % of ninhydrin in 95 % ethanol.

The possibility of the presence of carbohydrate was tested by using the modified Orcinol test (65). The phloroglucinol reaction was used for testing for aldopentoses (66). Inorganic phosphate (67) and total phosphate (68) were also tested.

Ultraviolet Spectrometry

The unknown compound was dissolved in deionized water and aliquots carefully adjusted to pH 1, 7, and 13 with HCl and NaOH. These solutions were subjected to ultraviolet spectra analysis by using a Beckman D. B. Spectrophotometer with a range of 100 MV, linear function and a chart speed of 2 inches per minute.

Gel Filtration

Sephadex G-50 gel was washed with water and was packed in a 2.0 X 45 cm column. A small amount of unknown sample in 5 ml of water, blue dextran with a molecular weight 2000 and NaCl were put on the column which was then eluted with water. Fractions of 4 ml were collected. Each fraction was subjected to the following measurements: Conductivity was determined by a type CDM conductivity meter. Absorbance at 660 mu was measured by Beckman D. B. Spectrophotometer. Radioactivity was measured by pipetting 0.1 ml of solution into 10 ml of scintillation

counter.

Paper Electrophoresis

Paper electrophoresis was carried out under the same conditions as described above but with initial and final temperature at 7°C.

CHAPTER IV

RESULTS AND DISCUSSION

Chromatographic Proof of Standard Homarine

Homarine standard solution and extracts of lobster muscle were chromatographed according to the precedure outlined in the section on Experimental Procedure. The Rf values of homarine isolated from lobster extracts as well as the standard homarine were 0.275, 0.572, and 0.236 with the solvent systems 95 % ethanol : NH_4OH (95 : 5, v/v), t-Butanol : NH_4OH : H_2O (60 : 30 : 10, v/v/v), and n-Butanol : Acetic Acid : H_2O (73 : 10 : 17, v/v/v) respectively.

Homarine was recovered from fractions 140-160 from the Dowex-50-H^+ column, when the column was eluted with different concentrations of HCl (Figure 1 and 2). With the Dowex-1- formate column, however, homarine was recovered in the deionized water fraction.

Determination of the Precursor to Homarine after Administration of Radioactive Compounds

Various carbon-14 labeled compounds were injected into lobsters and snails in an attempt to determine whether or not these compounds were precursor of homarine.

Methyl labeled homarine was also injected into the sea snail to determine whether this compound is metabolized by these invertebrates.

There are several possibilities for homarine synthesis in marine animals. Homarine could be synthesized from food sources, such as amino acids, carbohydrates, or fatty acids. Furthermore, some compounds such as glycerol, aspartic acid (44), acetate (42), and tryptophan (47) are known precursors of the pyridine ring in other organisms. For these reasons, various compounds, namely, aspartic acid, tryptophan, lysine, methionine, acetic acid, and glucose were injected into the lobsters.

There was also the possibility that homarine might act in one carbon transfer in marine invertebtates. It could either act in transmethylation or carboxylation or both. The possible transmethylation and transcarboxylation precursor compounds such as N-methylpyridine, methionine, formate, picolinic acid, and quinolinic acid were therefore also injected.

Unfortunately, none of the compounds mentioned above appears to be a precursor of homarine. This conclusion is based on the observation that no radioactive peak appeared near the homarine region on the Doxex-50-H^+ column. The result was further confirmed by the Rf values of the radioactive peaks from the Dowex-50-H^+ column which were all different from that of homarine in the solvent systems mentioned on page 21 .

When methyl labeled homarine was injected into the lobster and a tissue extract prepared 36 hours later, only one radioactive peak was isolated from the Dowex-50-H^+ column and this peak was identified as homarine.

Exeretion of respiratory $^{14}CO_2$ from the lobster and snail following the injection of some of the possible precursor compounds is shown in Table 1.

TABLE 1

Respiratory Carbon Dioxide from Marine Invertebrates after Injection of Radioactive Compounds

Animal	Compd. Inj.	Inj. Dose. (cpm)	Leng. of Exp. (hr.)	Resp. $^{14}CO_2$ (cpm)	% Recovery
Lobster	L-Lysine-2-^{14}C	5.7×10^7	54	1.11×10^7	19.4
Lobster	L-Aspartic Acid -U-^{14}C	5.3×10^7	36	1.7×10^7	32.1
Lobster	D,L-Tryptophan-Benzen Ring-^{14}C	5.8×10^7	36	3.9×10^5	0.67
Lobster	Quinolinic Acid-2,3,7,8-^{14}C	7.0×10^6	36	7.6×10^5	10.8
Snail	Homarine-Methyl-^{14}C	6.5×10^6	36	8.2×10^5	12.6
Snail	N-methylpyridine iodide-methyl-^{14}C	7.5×10^7	36	4.4×10^4	0.06

Haake and Mantecon (5) postulated a possible biological function of homarine in CO_2 transport. They proposed that homarine could be decarboxylated outside of the cell to form the N-methypyridinum ion, which could pass through the cell wall and be carboxylated inside the cell to form homarine. Carbon dioxide thus could be passed out of the body. This hypothesis suggests that homarine could act as a CO_2 storage sink. It might also be involved in active transport of ions out of the cell.

From the results presented above, the methyl group of homarine is removed to form CO_2. On the other hand, failure to demonstrate the presence of a methyl pyridine peak on the Dowex-50-H^+ column, or on paper chromatography suggests that Haake's hypothesis is not correct. The biological function of homarine in marine invertebrates is still unknown.

Characterization of an Unknown Metabolite Formed from Methionine in the Lobster

Methyl labeled methionine-^{14}C was injected into a living lobster to determine whether or not it is a precursor of homarine. On Dowex-50-H^+ chromatography of the tissue extract a radioactive peak was observed very close to the homarine region, in fractions 175 to 190, as shown in Figure 5. This compound could be eluted with water from the Dowex-1-formate column. Although the compound was located very close to homarine on column chromatography, it was not homarine for it had a Rf value of 0.125 whereas

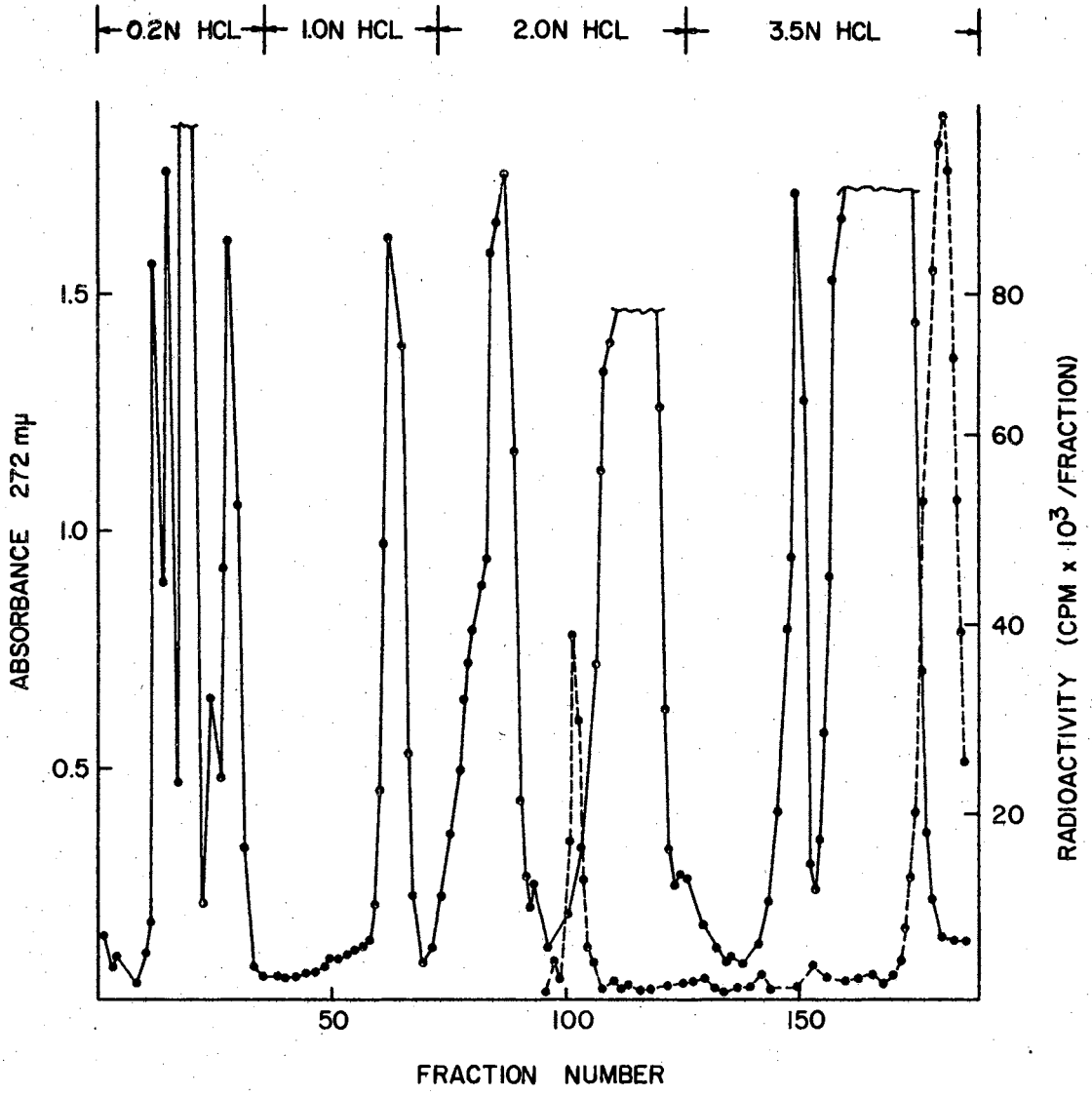

Figure 5. Chromatographic Separation of a Metabolite of Methionine from Lobster Muscle Extract on a Dowex-50-H⁺ Cation Exchange Column

·----------· Radioactivity
.__________. Ultraviolet absorption

homarine and methionine have Rf values of 0.284 and 0.418 respectively in a solvent system of n-Butanol : Acetic Acid : H_2O (60 : 20 : 20, v/v/v) on decending paper chromatography.

Since this unknown was ninhydrin positive, the compound was suspected to be a small peptide or an unknown amino acid. For further characterization, the compound was applied to an amino acid analyzer using the physiological column. Several unknown peaks were observed in the amino acid chromatogram as shown in Figure 6B, but except for a small amount of methionine there was only one peak, from 60 to 70 minutes elution time which contained radioactivity. This radioactive peak was located before the aspartic acid peak of an standard amino acid analysis chromatogram as shown in Figure 6A.

The paper electrophoretic mobility of this compound under the conditions described in Experimental Procedure was +1.1 cm.

The unknown compound did not show any maxima or minima absorption between the wave lenth 740 to 220 mu on a Beckman D. B. Spectrophotometer. These results suggest the unknown compound may be a peptide containing methoinine.

Characterization of an Unknown Metabolite formed from Picolinic Acid in the Lobster

After ninty six hours injection of 2.56 uc (232.7 uM) of radioactive picolinic acid into a lobster, the

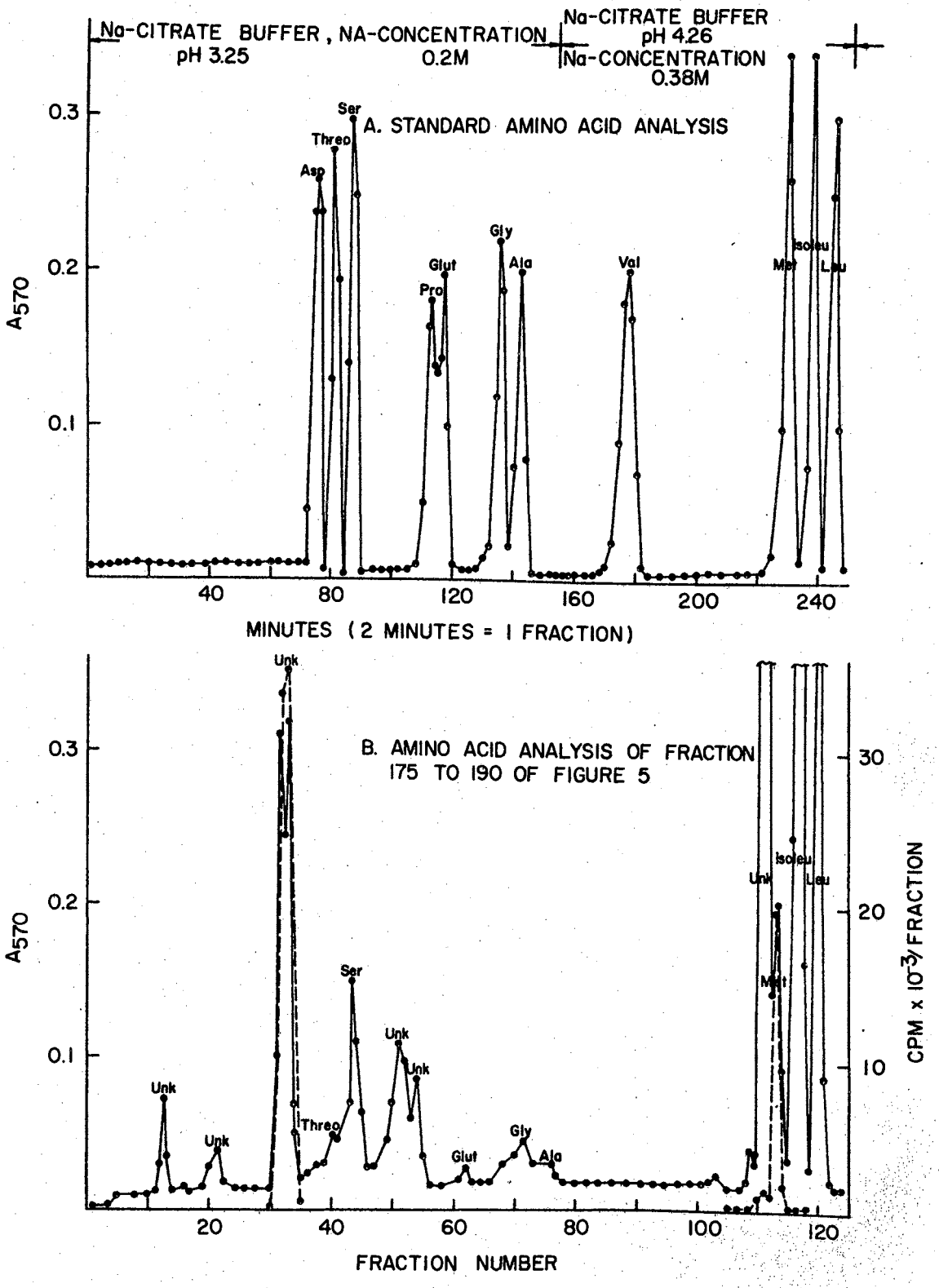

Figure 6: Analysis of Amino Acids and Unknown Metabolites of Methionine with a Beckman-Spinco Amino Acid Acid Analyzer Model 120.
._____.Ninhydrin Color Yield
.----------.Radioactivity

tissue extract was put on Dowex-50-H^+ column to determine whether or not picolinic acid is a precursor of homarine. Several radioactive peaks were present in the chromatogram. The peak which contained the most radioactivity was from fraction number 23 to 27 (Figure 7).

The further purification of this compound was accomplished with an anion exchange Dowex-1-formate column as shown in Figure 8. The compound could be eluted from the column with 6 N formic acid.

The unknown compound did not react with ninhydrin or the Fe $(NH_4)_2$ $(SO_4)_2$ reagent to give a color test. This compound was not separated by this column chromatographic procedure from a contaminating nucleotide compound. Therefore, the radioactive peak from the Dowex-1-column gave an analysis of carbohydrate and one mole of phosphate for every two moles of unknown (Table II). The concentration of unknown was determined by using the extinction coefficient of picolinic acid because of the similarity of the ultraviolet spectra at different pH values of both compounds. The contaminating nucleotide could be separated by thin layer or paper chromatography by using the solvent system mentioned in the Experimental Procedure. The purified unknown compound did not contain phosphate or carbohydrate (Table II). The ultraviolet spectra of the unknown and of standard picolinic acid at 3 different pH values are shown in Figures 9 and 10.

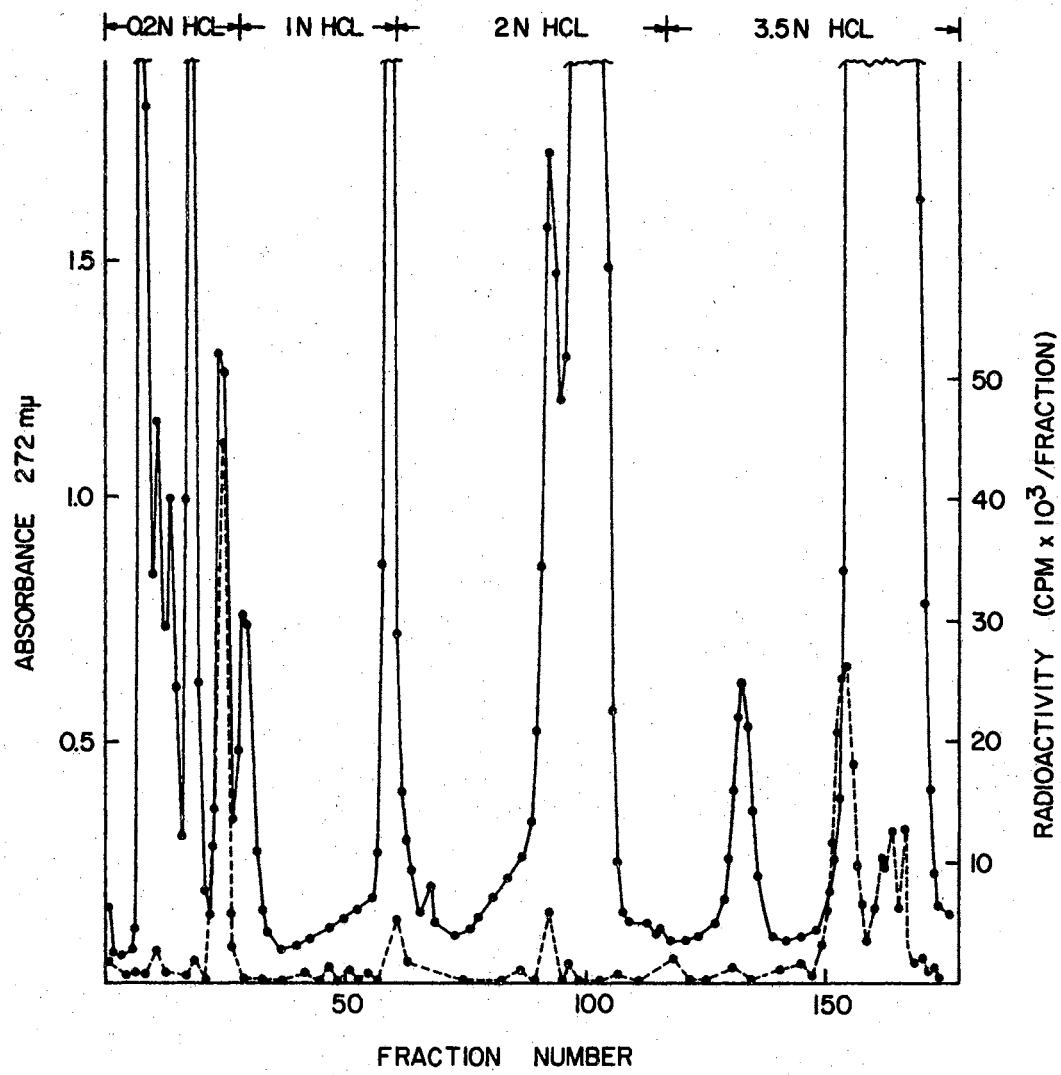

Figure 7. Chromatographic Separation of an Unknown Metabolite of Picolinic Acid from Lobster Muscle Extract on a Dowex-50-H^+ Cation Exchange Column

._____.UV absorption
.----------.Radioactivity

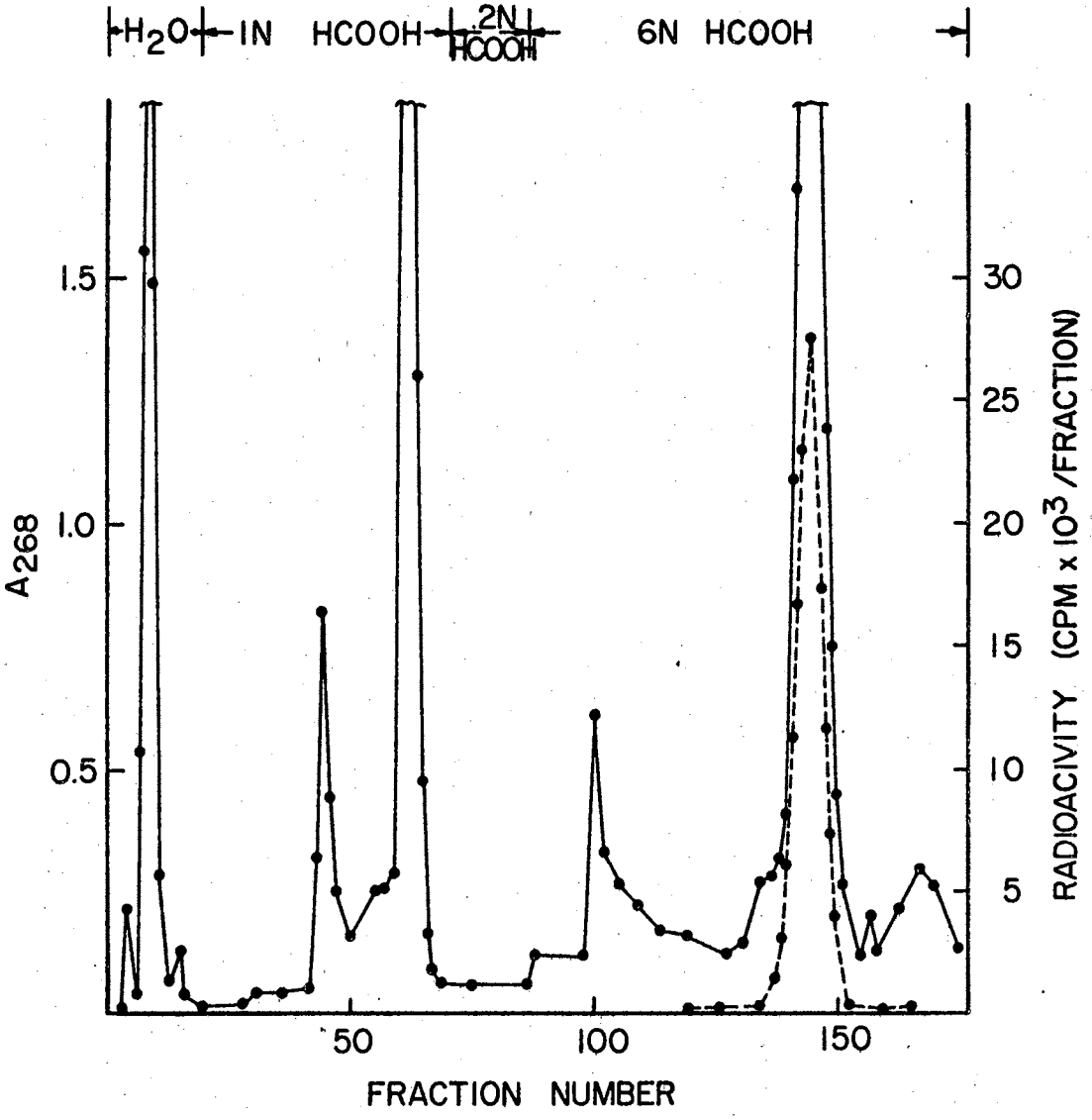

Figure 8. Purification of the Unknown Metabolite of Picolinic Acid on a Dowex-1-Formate Column

._____. Ultraviolet absorption
.----------. Radioactivity

TABLE II

Phosphate and Carbohydrate Content of the Unknown Picolinic Acid Metabolite before and after Purification by Silica Gel Thinlayer Chromatography Using the Solvent System n-Butanol : Acetic Acid : H_2O (73 : 10 : 17, v/v/v)

	Before Purification	After Purification
Amount of Unknown Tested uM	0.37	0.37
Carbohydrate content uM	0.32	0
Amount of unknown Tested uM	0.085	0.05
Phosphate Content uM	0.090	0

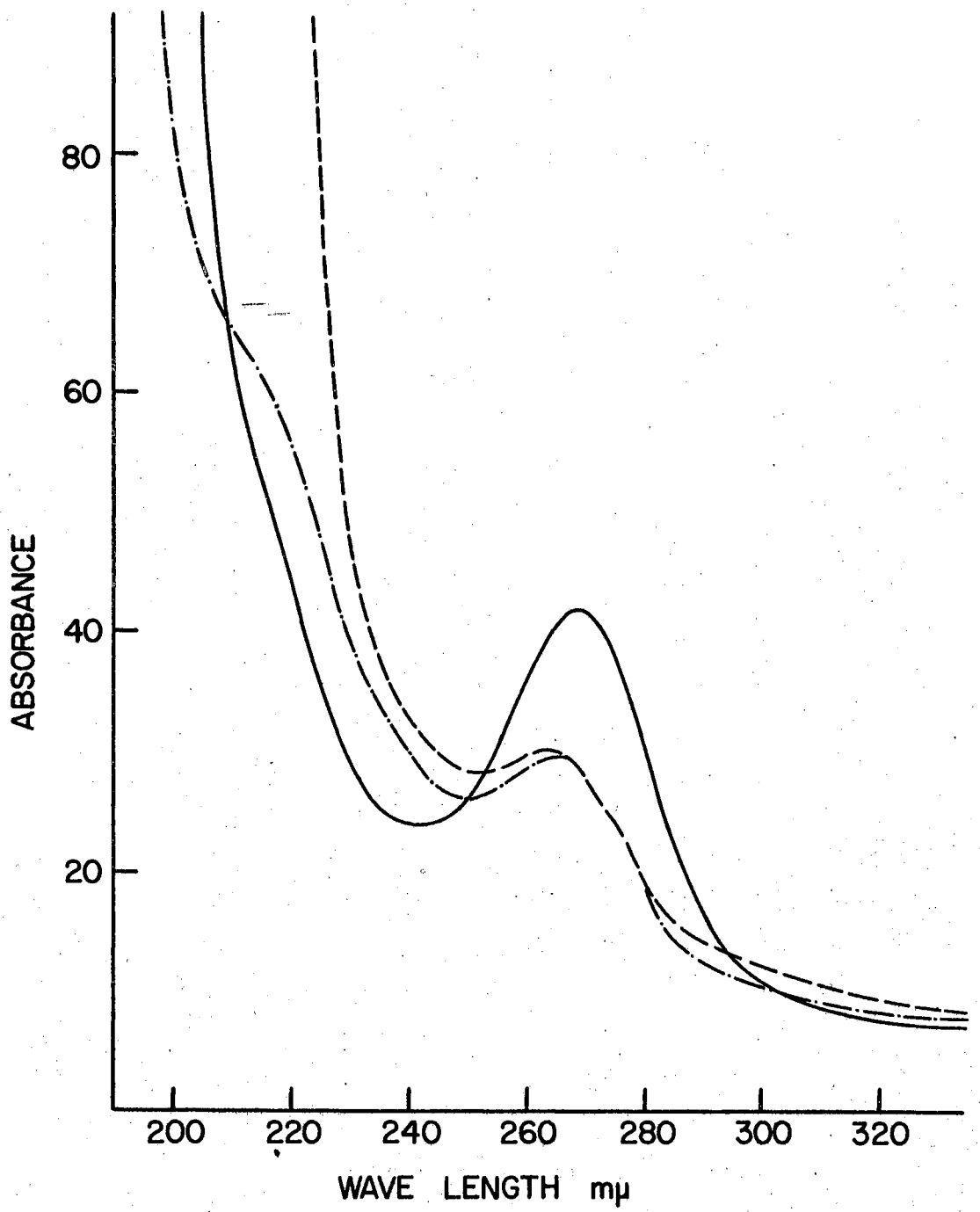

Figure 9. The Ultraviolet Spectra of the Unknown Picolinic Acid Metabolite at Different pH Values

_____pH 1.0
----------pH 13.0 .-.-.- pH 7.0

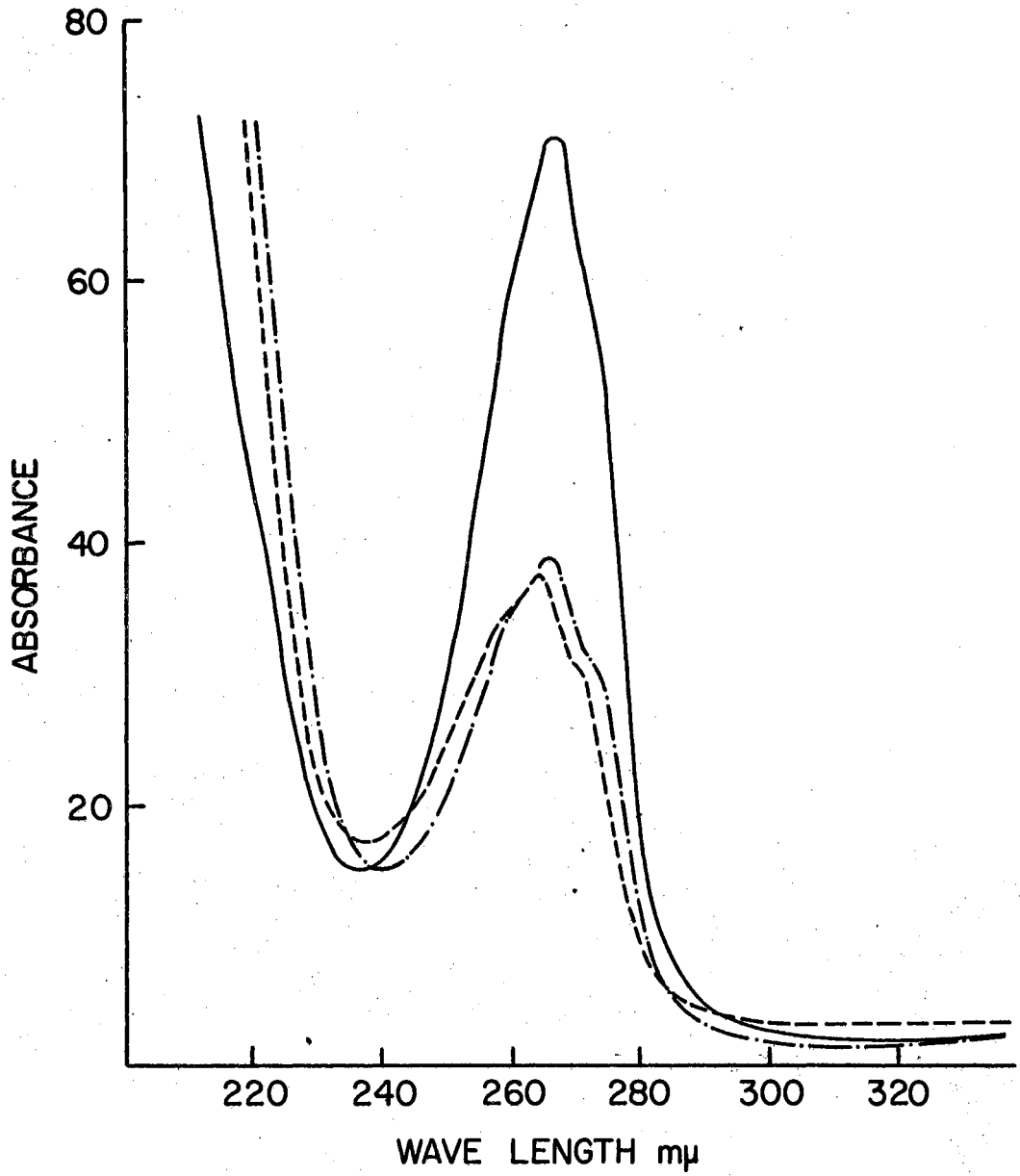

Figure 10. The Ultraviolet Spectra of Standard Picolinic Acid at Different pH Values
_____ pH 1.0 --------
pH 13.0 .__.__. pH 7.0

From the results of gel filtration, the molecular weight of the compound is less than 2,000. This was shown (Figure 11) by the fact that the unknown compound was eluted from the column after the blue dextran peak.

Paper electrophoresis at pH 5.0 showed a mobility value of +2.2 cm for the unknown whereas quinolinic acid had a value of +5 cm, nicotinic acid +1.5 cm, and picolinic acid +0.7 cm. These results showed that the unknown compound has a total of net negative charge 1 or 2.

It is known that picolinic acid is an end product of tryptophan metabolism and is excreted essentially quantitatively as its glycine conjugate (6, 39) in rats. An amino acid analysis on the compound hydrolyzed in 6 N HCl in vacuuo for 36 hours showed no glycine content in the compound. This finding eliminated the possibility that the unknown is the unknown is the glycine conjugate of picolinic acid. The author did not find any reports on the metabolism of picolinic acid in marine invertebrates.

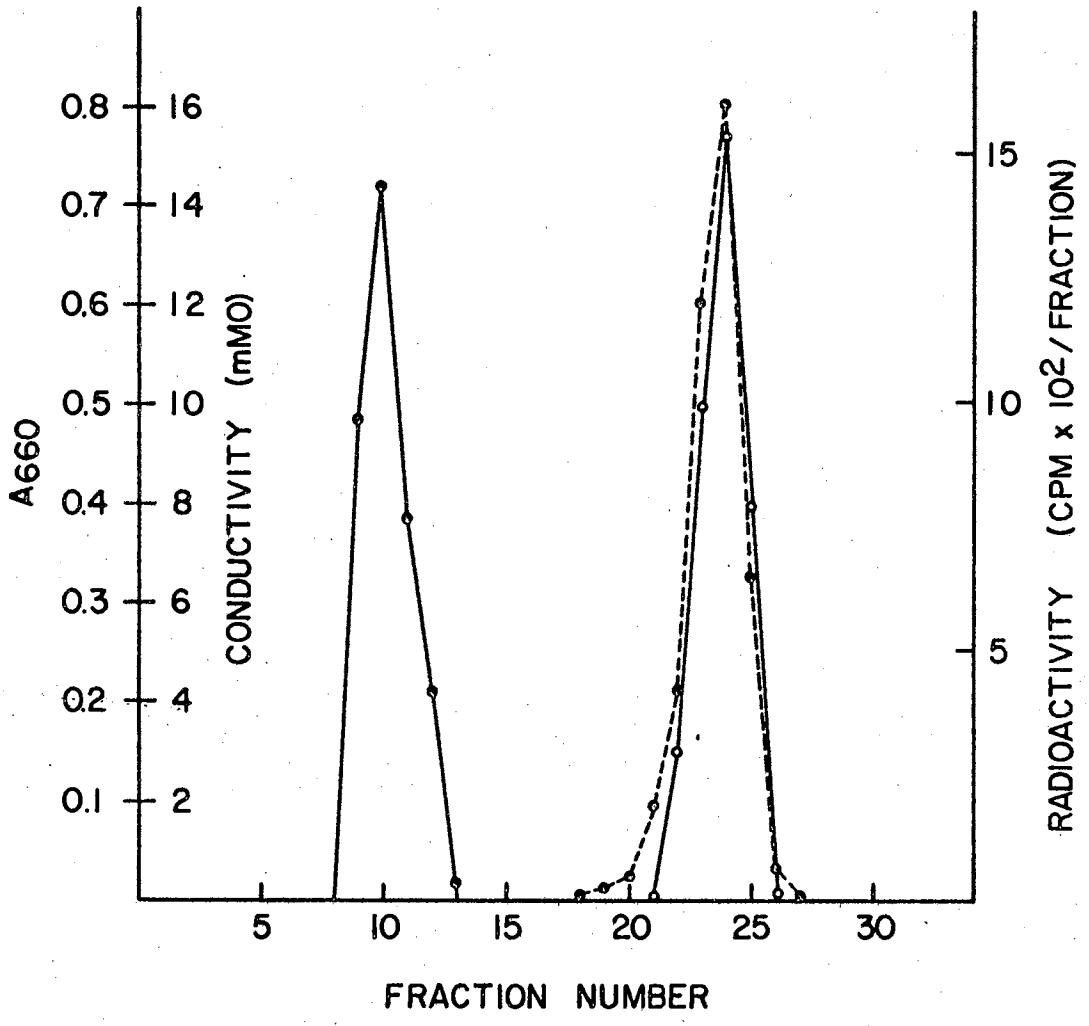

Figure 11: Gel Filtration (Sephadex G50) of the Unknown Picolinic Acid Metabolite with Column Size 2.0 X 45 cm and Eluted with H_2O

. _____ . Absorbance
. - - - - - - - - - - . Conductivity
o _____ o Radioactivity

SUMMARY

Radioactive aspartic acid, lysine, tryptophan, acetate, quinolinic acid, glucose, methionine, sodium formate, picolinic acid, and N-methylpyridine did not give rise to detectable amounts of homarine when injected into lobsters and snails. Carbon dioxide-^{14}C was evolved after homarine-methyl-^{14}C was injected into lobsters.

An unknown metabolite was formed after picolinic acid injection. This unknown metabolite was ninhydrin negative and could be eluted from a Dowex-50-H^+ column with 0.2 N HCl, and from a Dowex-1-formate column with 6 N formic acid. The compound has a migration of +2.2 cm on paper electrophoresis in pH 5.0 buffer under conditions such that picolinic acid had a migration of +0.7 cm, nicotinic acid +1.5 cm, and quinolinic acid +5.0 cm.

The ultraviolet spectrum is different from that of picolinic acid by the shift in its minimum absorption wave lenth from 250 to 240 upon a pH shift from 7.0 to 1.0 in the new metabolite.

An unknown metabolite was formed from methionine in the lobster. This unknown compound was ninhydrin positive, and could be eluted from a Dowex-50-H^+ column with 3.5 N HCl and from a Dowex-1-formate column with water. On

electrophoresis it showed a migration +1.1 cm in pH 5.0 buffer under conditions at which picolinic acid, nicotinic acid and quinolinic acid had migrations of +0.7, 1.5 and 5.0 cm respectively.

LITERATURE CITED

1. Hoppe-Seyler, F. A., Z. Physiol. Chem., 222, 105 (1933)

2. Gasteiger, E. L., Gergen, J., and Haake, P., Biol. Bull., 109, 345 (1955)

3. Gasteiger, E. L., Haake, P. C., and Gergen, J. A., Ann. N. Y. Acad. Sc., 90, 622 (1960)

4. Levy, R. A., Comp. Biochem. Physiol., 23, 631 (1967)

5. Haake, P., and Mantecon, J., J. Am. Chem. Soc., 86, 5230 (1964)

6. Mehler, A. H., and May, E. L., J. Biol. Chem., 223,

7. Dagley, S., and Johnson, P., Biochim. Biophy. Acta, 78, 577 (1963)

8. Holtz, F., Kutscher, F., and Thielmann, F., Z. Biol., 81, 57 (1924)

9. Haurowitz, F. and Waelsch, H., Z. Physiol. Chem., 161, 300 (1926)

10. Kutscher, F., and Ackermann, D., Z. Physiol. Chem., 221, 33 (1933)

11. Mii, S., J. Chem. Soc. Japan, 63, 753 (1942)

12. Welsh, J. H., and Prock, P. B., Biol. Bull. Mar. Lab. Woods Hole, 115, 551 (1958)

13. Mathias, A. P., Ross, D. M., and Schachter, M., J. Physiol., 151, 296 (1960)

14. Keyl, M. J., Michaelson, I. A., and Whittaker, V. P., J. Physiol., 139, 434 (1957)

15. Leonard, G. J., and Macdonald, K., Nature, 200, 78 (1963)

16. Nishita, Kiyoyoshi, Arai, and Saito, T., Bull. Fac. Fish Hokkaido Univ. 16, 114 (1965)

17. Ackermann, D., and Menssen, H. G., Z. Physiol. Chem., 317, 114 (1949)

18. _____, Physiol. Chem., 295, 1 (1953)

19. _____, and Janka R., Hoppe-Seyler's Z. Physiol. Chem., 298, 65 (1954)

20. _____, Hoppe-Seyler's Z Physiol. Chem., 302, 80 (1955)

21. _____, and List, P. H., Z. Physiol. Chem., 306 260 (1957)

22. _____, Z. Physiol. Chem., 313, 30 (1958)

23. Ackermann, D., and Pant, R., Z. Physiol. Chem., 326, 197 (1961)

24. List, P. H., Planta Med., 6, 424 (1958

25. List, P. H., and Menssen, H, G., Arch. Pharm., 292, 260 (1959)

26. Kalckar, J. U., Strominger, J. L., and Gevirtz, N. R., Biol. Bull., 105, 391 (1953)

27. Hultin, T., Lindvall, S., and Gustafsson, K., Arkiv. Kemi., 6, 477 (1953)

28. Deffner, G. G., and Hafter, R. E., Biochim et Biophy. Acta, 42, 189 (1960)

29. Koechlin, B. A., J. Biophy. Biochem. Cytol., 1, 511 (1955)

30. Gasteiger, E. L., Federation Proc., 12, 1 (1953)

31. Fukushima, Nippon Suissan Gakkaishi, 28, 808 (1962)

32. Riegel, B., Stanger, D. W., Wikholm, D. M., Mold, J. D., and Sommer, H., J. Biol. Chem., 177, 1 (1949)

33. Schantz, E. J., Amer. Chem. Soc. Abstract, 156, AGRD 20. (1968)

34. Kravitz, E. A., Kuffler, S. W., Potter, D. D., and Gelder, N. M., J. Neurophysiol., 26, 729 (1963)

35. Tsukamoto, T., and Komori, T., Pharm. Bull., 3, 243 (1955)

36. Ciusa, W., and Barbiroli, G., Ann. Chim, 53, 1516 (1963)

37. Mayer, A. W., and Vigneaud, V., J. Biol. Chem., 143 373 (1942)

38. Joshi, J. G., and Handler, P., J. Biol. Chem., 237, 3185 (1962)

39. McKennis, H., Bowman, E. R., Horvath, A., and Bederka, J. P., Nature, 202, 669 (1964)

40. Griffith, R., and Byerrum, R. U., Biochem. and Biophy Comm., 10, 293 (1963)

41. Friedman, A. R., and Leete, E. J., J. Am. Chem. Soc., 85, 2141 (1963)

42. Griffith, T., Hellman, K. P., and Byerrum, R. U., Biochem., 1, 337 (1962)

43. Jackanicz, T. M., and Byerrum, R. U., J. Biol. Chem., 241, 1296 (1966)

44. Yang, K. S., Gholson, R. K., and Waller, G. R., Am. Chem. Soc., 87, 4184 (1965)

45. Ahmad, F., and Moat, A., J. Biol. Chem., 241, 775 (1966)

46. Mehler, A. H., J. Biol. Chem., 218, 241 (1955)

47. Mitchell, H. K., and Nye, J. F., Proc. Nat. Acad. Sc., 34, 1 (1948)

48. Powell, J. F., Biochem. J., 54, 210 (1953)

49. Perry, J. J., and Foster, J. W., J. Bacteriol., 69, 337 (1955)

50. Martin, H. M., and Foster, J. W., J. Bacteriol., 76, 167 (1958)

51. Gilvarg, C., J. Biol. Chem., 236, 1429 (1961)

52. Peterkofsky, B., and Gilvarg, C., J. Biol. Chem., 236, 1432 (1961)

53. Yugari, Y., and Gilvarg, C., J. Biol. Chem., 240, 4710 (1965)

54. Tanenbaum, S. W., and Kaneko, K., Biochemistry, 3, 1314 (1964)

55. Bach, M. L., and Gilvatg, C., J. Biol. Chem., 241 4563 (1966)

56. Prescott, A. B., J. Am. Chem. Soc., 18, 91 (1896)

57. Schenk, W., Z. Chem., 2, 115 (1962)

58. Pollini, V., Vasconetto, C., and Ricci, C., Boll. Soc. Ital. Biol. Sper. 41, 673 (1965)

59. Waller, G. R., Proc. Okla. Acad. Sci., 47, 271 (1968)

60. Li, H. T., Walden, J., Etter, D., and Waller, G. R., Proc. Okla. Acad. Sci., 49, (1968) (In Press).

61. Badiei, S., Thesis of Oklahoma State University, (1968)

62. Prudden, T. M., About Lobsters, The Bond Wheelwright Co., Freeport, Maine, 1962, p. 91

63. Leifer, E., Roth, L. J., Hogness, D. S., and Carson, M. H., J. Biol. Chem., 190, 595 (1951)

64. Waller, G. R., and Henderson, L. M., J. Biol. Chem., 236, 1186 (1961)

65. Dische, Z., J. Biol. Chem., 204, 984 (1953)

66. Dische, Z., and Borenfreund, E., Biochim. Biophy. Acta, 23, 639 (1957)

67. Chen, P. S., Toribara, T. Y., and Warner, H., Anal. Chem., 28, 1756 (1956)

68. Ames, B. N., and Dubin, D. T., J. Biol. Chem., 235, 769 (1960)

VITA

2

Louise Niyine Chu

Candidate for the Degree of

Master of Science

Thesis: STUDIES ON THE BIOSYNTHESIS OF HOMARINE AND THE CATABOLISM OF PICOLINIC ACID IN HOMARUS AMERICANUS AND BUSYCON FULGAR

Biographical:

Personal Data: Born in Shanghai, China, November 25, 1933, the daughter of Yin-Song and Han-Fong Chu.

Education: Graduate from Taiwan Provincial Taichung Girl's High School, Taichung, Taiwan in 1953; received the bachelor of science degree from the Taiwan Provincial Chung-Hsing University, Taichung, Taiwan in Horticulture in June 1957; received the master of science degree from Texas Woman's University, Denton, Texas in Nutrition in May, 1963; complete the requirements for the master of science degree in May 1970.

Professional Experience: Served as a research assistant in plant breeding and cytogenetics in Taiwan Provincial Agricultural Research Station, Taipei, Taiwan, 1957 to 1958; served as a lecturer in Taiwan Provincial I-Lan, Taiwan, 1958 to 1959; served as an inspector in vegetables and fruits in Taiwan Provincial Bureau of Inspection, Taipei, Taiwan, 1959 to 1961; served as research assistant in Texas Women's University, 1961 to 1963; served as a research assistant in University of California, 1963 to 1965; served as research assistant in Oklahoma State University, 1967 to 1969.